

# *AMINO ACIDS*

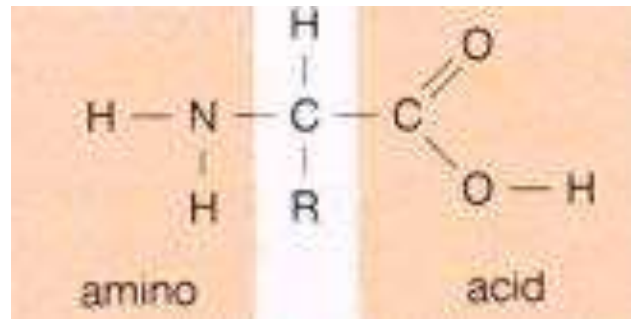
## *[QUALITATIVE TESTS]*

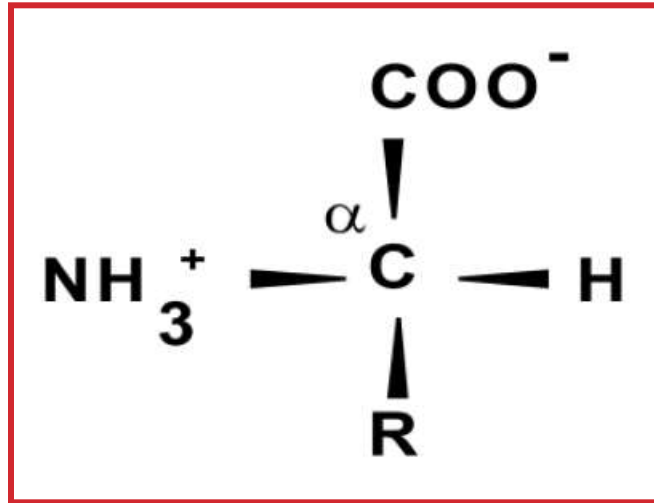
## Amino acids:

-Building blocks of proteins.

-Intermediates in metabolism.

-There are **20** natural amino acids that are found within proteins . **All of them are L- $\alpha$  amino acids.**

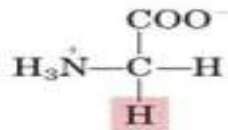




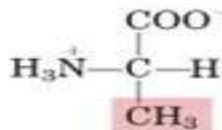
General structure of amino acids

- All amino acids found in proteins have this basic structure, differing only in the **structure of the R-group** or the side chain.
- The simplest, and smallest, amino acid found in proteins is glycine for which the R-group is hydrogen (H).

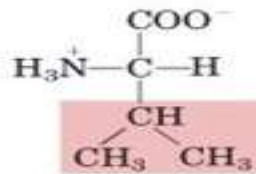
### Nonpolar, aliphatic R groups



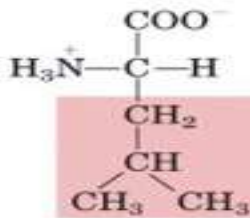
Glycine



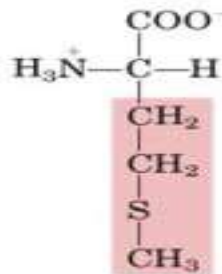
Alanine



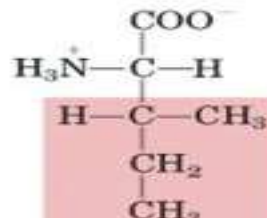
Valine



Leucine

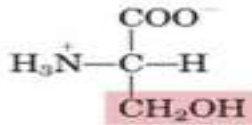


Methionine

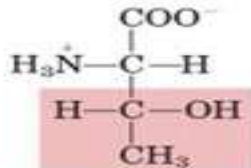


Isoleucine

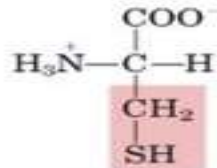
### Polar, uncharged R groups



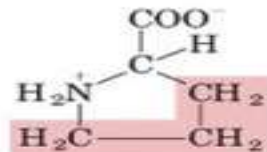
Serine



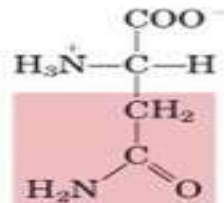
Threonine



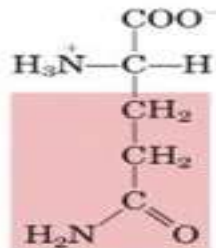
Cysteine



Proline

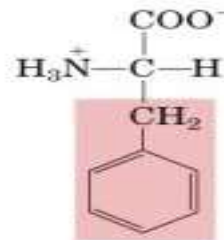


Asparagine



Glutamine

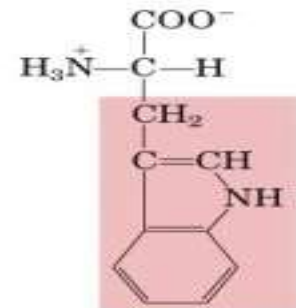
### Aromatic R groups



Phenylalanine

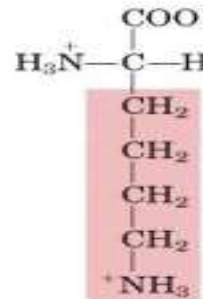


Tyrosine

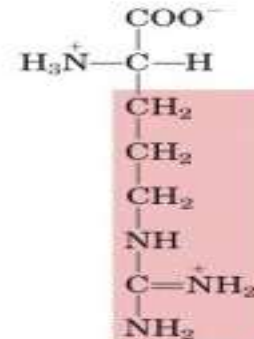


Tryptophan

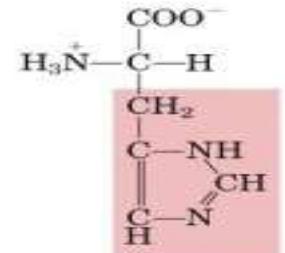
### Positively charged R groups



Lysine

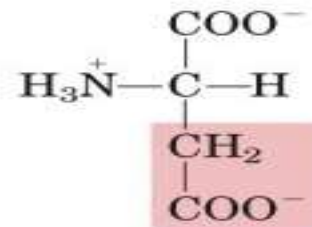


Arginine

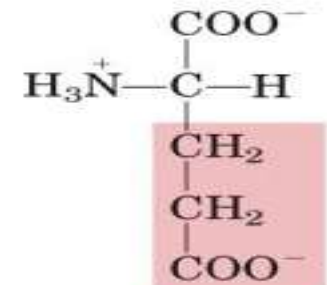


Histidine

### Negatively charged R groups



Aspartate



Glutamate

## Classification of amino acids:

According to their ionization (polarity) in water, they are classified into 4 categories:

- 1- Non-polar.
- 2- Uncharged polar.
- 3-polar amino acids :
  - A-Basic polar (positively charged).
  - B- Acidic polar (negatively charged).

### Note:

**Polar amino acids are more soluble in water** than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution, and therefore, to act as acids and bases.

## Some properties of Amino Acids:

### 1- Amphoteric Compounds:

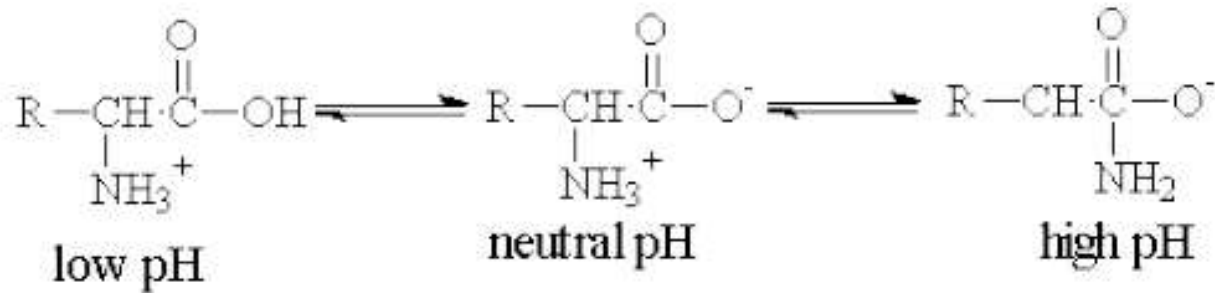
which mean they can act as acids and bases.

Due to presence of carboxyl group COOH that able to **donate** proton( $H^+$ ), and convert to  $COO^-$ .



Also presence of amino group  $NH_2$  which is enable to **accept** this proton( $H^+$ ) and convert into  $NH_3^+$





**Amino acids are amphoteric Compounds**

# Practical part

1. Solubility test.

2. Ninhydrin test: for  $\alpha$ -L amino acids.

3. Xanthoproteic test: for Aromatic amino acids.

4. Millon's test: for amino acids containing hydroxylphenyl group

5. Sakaguchi Test.

6. Lead sulfite test: detection of amino acids containing sulfhydryl group (- SH)



# 1.Solubility test:

## Objective:

investigate the solubility of selected amino acid in various solutions

## Principle:

Polar amino acids are more soluble in water[polar] than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution.

Polar amino acids are soluble in polar solvent, and vice versa.



## 2. Ninhydrin test:

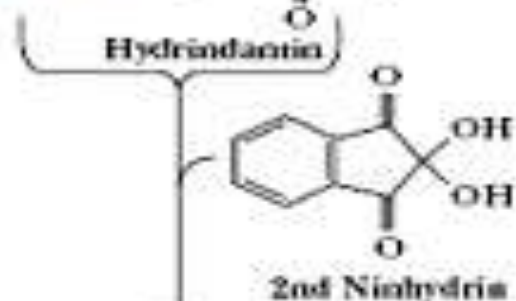
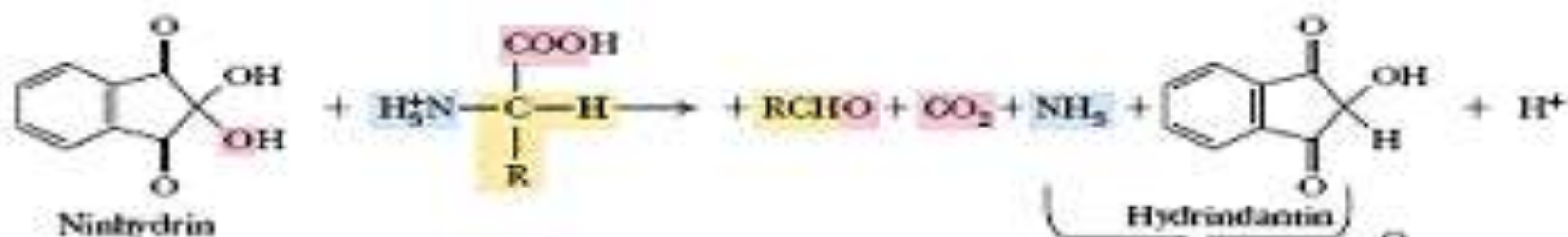
### Objective:

-to detect  $\alpha$ -L-amino acids

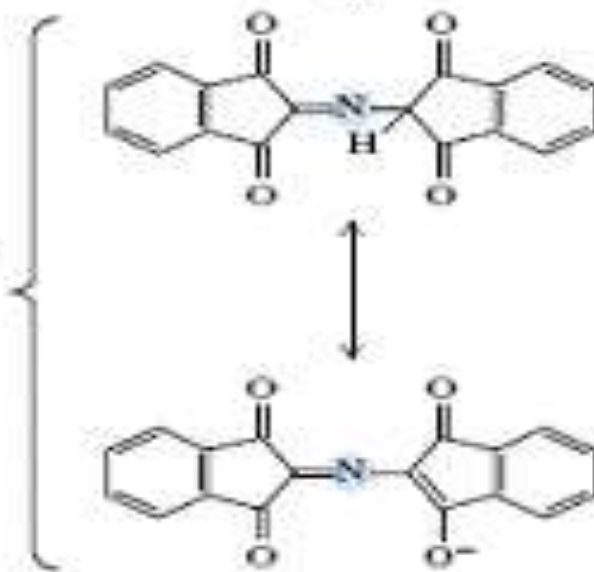
### Principle:

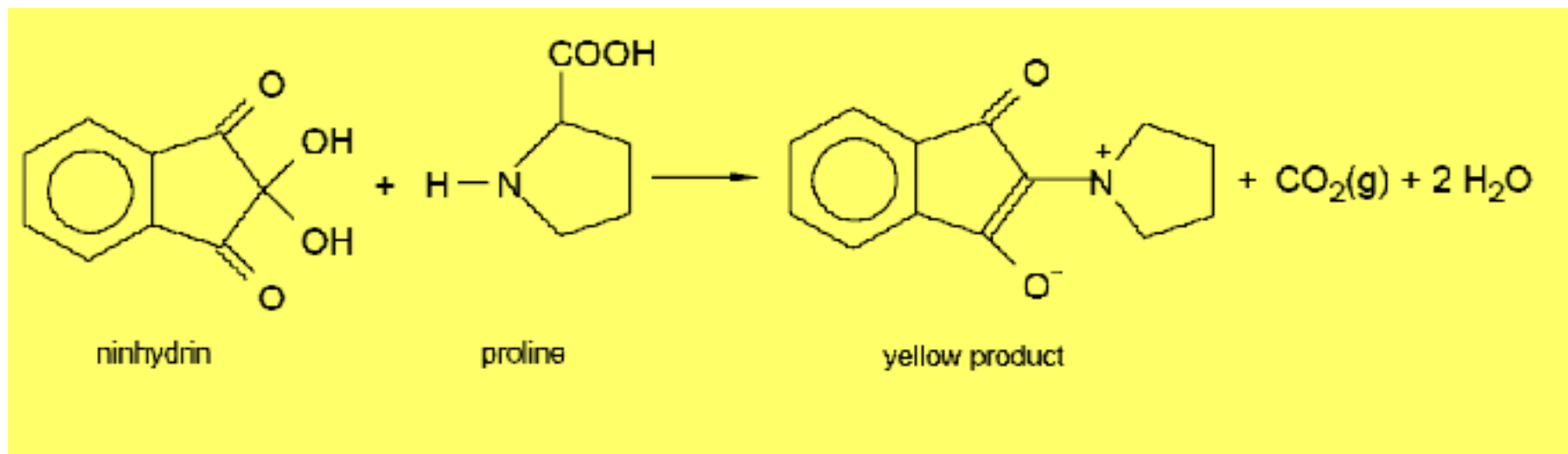
1. Ninhydrin (triketohydrindene hydrate) degrades amino acids into aldehydes (on pH range 4-8), ammonia and CO<sub>2</sub> through a series of reactions.

2. Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely **blue or purple pigment**, sometimes called Ruhemann's purple



Two resonance forms of Ruhemann's Purple





All amino acids that have a free amino group will give positive result (**purple color**) .

While not free amino group-proline and **hydroxy-proline** (amino acids) will give a (**yellow color**).

**Note:** Many substances other than amino acids, such as **amines** will yield a **blue color** with ninhydrin, particularly if reaction is carried out on filter paper.



### 3.Xanthoproteic test :

#### Objective:

to differentiate between aromatic amino acids which give positive results [yellow color] and other amino acids.

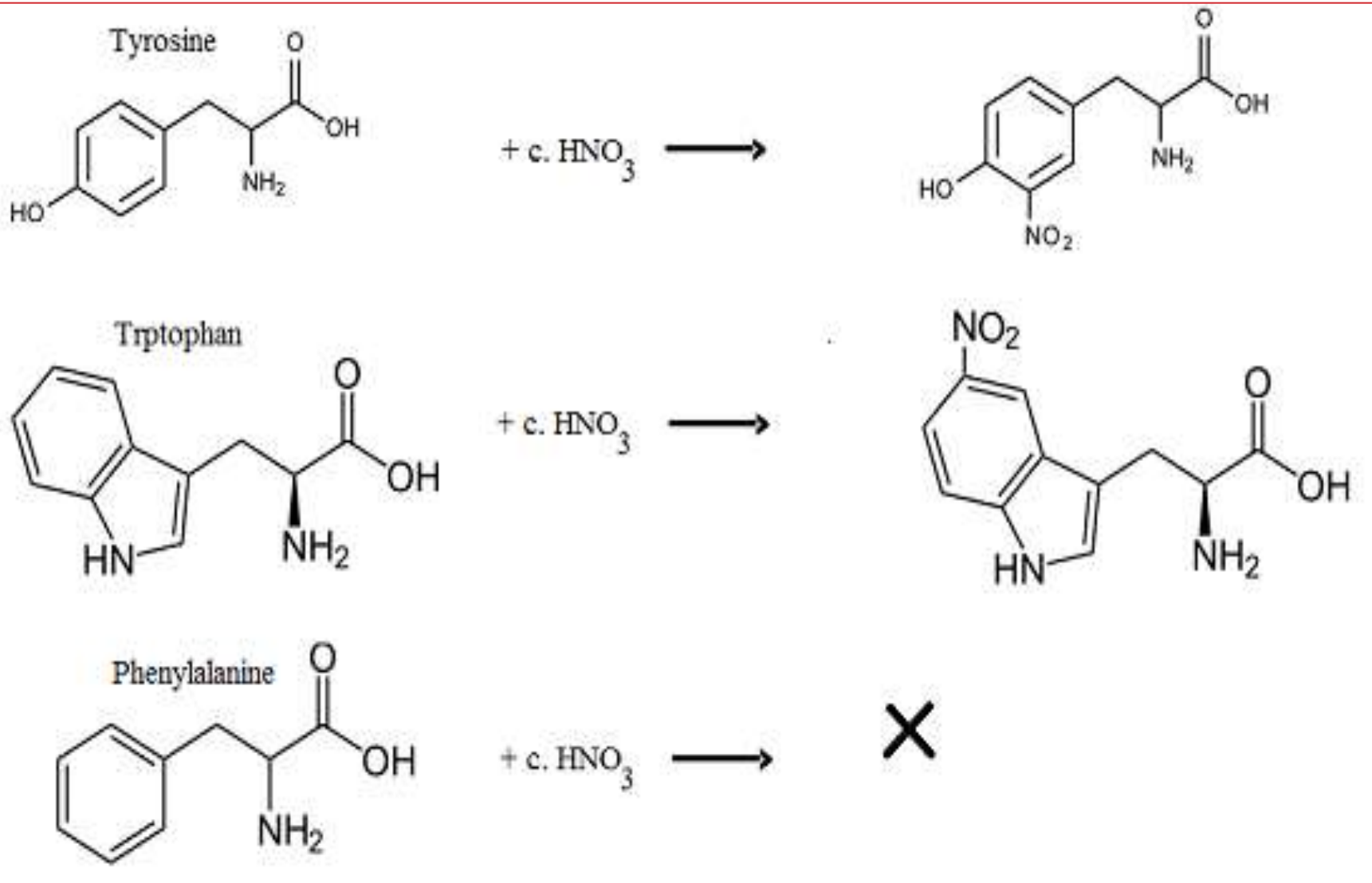
#### Principle:

Concentrated nitric acid react with aromatic nucleus present in the amino acid side chain [nitration reaction] → giving the solution yellow color.

#### Note:

Amino acids **tyrosine and tryptophan** → contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow colored compounds.

The aromatic ring of **phenyl alanine** **dose not react** with nitric acid despite it contains a benzene ring, but it is **not activated**, therefore it will not react



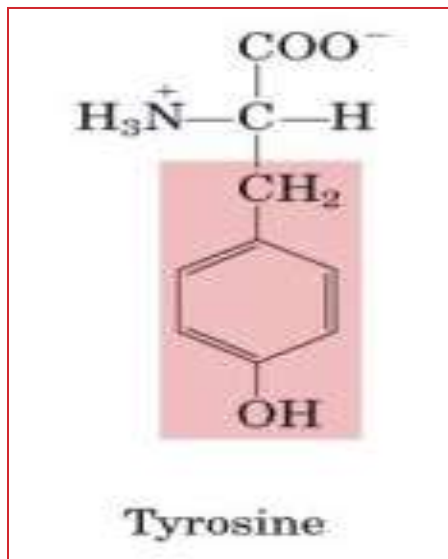




## 4. Millon's test:

### Objective:

This test is specific for tyrosine. Because it is the only amino acid containing a phenol group.



**Note:** phenol group, a hydroxyl group attached to benzene ring.

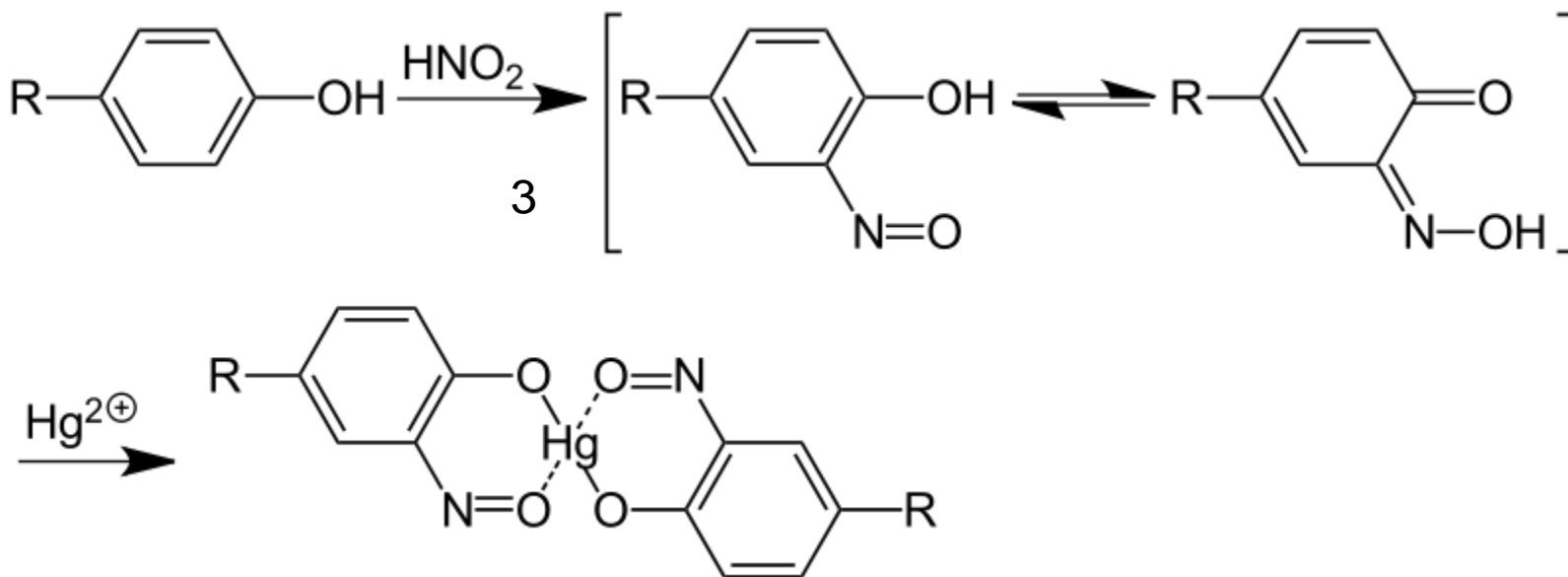


## Principle:

The phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury ions in the solution to form a **brick-red**, appearance of **red color is positive test**.

## Note:

all phenols (compound having benzene ring and OH attached to it) give positive results in Millon's test.



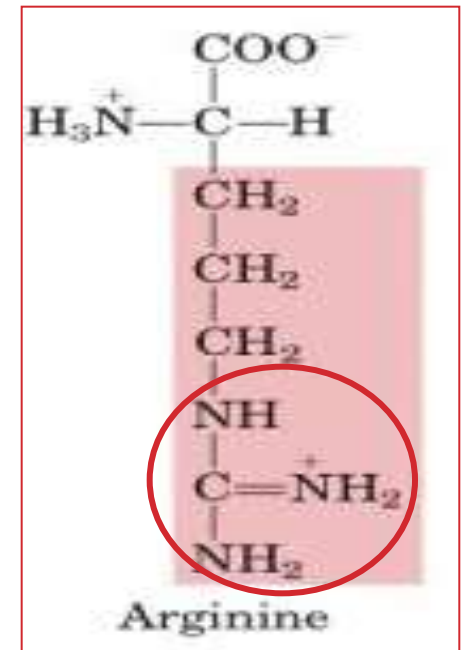
## 6. Sakaguchi Test:

### Objective:

detection of amino acid containing guanidinium group. In other words it's a test for, arginine.

### Principle:

In **alkaline** solution, arginine react with  $\alpha$ -naphthol and sodium hypobromite /chlorite as an oxidize agent, to form red complexes as a positive result.





## 6. Lead Sulfite Test:

### Objective:

This test specific for **-SH [sulfhydryl group]** containing amino acid (**Cysteine**).

### Principle:

- Sulfur in cystine, is converted to sodium sulfide by boiling with 40% NaOH.
- The Na<sub>2</sub>S can be detected by the precipitation of PbS (lead sulfide) from an alkaline solution. when adding lead acetate Pb (CH<sub>3</sub>COO)<sub>2</sub>.

